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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

We are currently investigating a putative tumor suppressor gene (TSG) located at chromosomal band 8p22 and its potential gene targets that are involved in breast cancer. We previously proposed to apply the genetic suppressor element (GSE) approach to the identification of this TSG. Briefly, a library of short gene fragments will be introduced into a cell line which demonstrates suppression of clonogenicity in soft agar with the transfer of chromosome 8. Presumably, the 8p22 TSG is responsible for the suppression of clonogenicity, and the introduction of an "active" GSE from the library into the suppressed cells should inhibit the 8p22 TSG contained in the hybrid cells and allow reversion back to the parental phenotype, the ability to grow in soft agar. Any clones that form will be isolated and further evaluated, as a candidate for the TSG. A complete list of ESTs were identified in the 8p22 interval using various methods. However, further characterization and analysis of these transcripts has led to the realization that these sequences do not represent real genes, and most likely are not expressed. Thus while our original objective of tumor suppressor gene identification remains the same, we have revised our strategy towards this end. We have identified genes that are differentially expressed between our parental/hybrid model, and are focusing on these genes as targets for further study. They represent genes that are potentially involved in suppression of malignant characteristics in breast cancer cells, and may eventually be used diagonostic, therapeutic, and/or prognostic use.

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Introduction

Breast cancer is generally characterized as a number of pathologically distinct entities that constitute the progression of lesions from hyperplasia, to carcinoma in situ, to invasive carcinoma, finally resulting in disease metastasis (1). Tumor suppressor genes (TSG) have been known to play an important role in the initiation and progression of human cancers, and there exists a large body of data that suggests the existence of a TSG on the short arm of chromosome 8 (8p). 8p is of particular interest in breast cancer because it is a site of frequent allelic loss in breast tumors, and it has been shown that 8p loss is one of the most common events in breast cancer (2). However, 8p loss is seen in a wide range of epithelial carcinomas (3-7), and is thought to be non tissue-specific. Several regions of 8p have been identified by LOH as putative TSG sites, including 8p22, where a homozygous deletion was reported in prostate cancer (8). Since homozygous deletions are regarded as the most sensitive indicator of TSG presence, we have chosen to concentrate our efforts in this region. Furthermore, others in our lab have shown functional evidence that chromosome 8 harbors tumor suppressor activity through monochromosome 8 transfer into cell lines derived from breast tumors, resulting in a decrease in tumorigenicity and clonogenicity in soft agar (unpublished). These matched cell line pairs offer an in vitro phenotype that is easily scorable and provides a model assay system. Using this model, we originally proposed to use a functional negative selection approach to identify a tumor suppressor gene on 8p that is involved in breast carcinoma. In brief, we proposed to construct a library of genetic suppressor elements (GSE), which consists of short gene fragments which encode inhibitory sense or antisense RNA (9-10), and introduce this library into our suppressed chromosome 8 containing breast cancer cell lines, as a means of identifying the suppressive genes. Any active GSEs will inhibit the function of the putative TSG on 8p in these suppressed lines and as a result, "unsuppress" the cell clone, thus allowing colony formation in soft agar. Isolation, expansion, identification, and verification of the clones will follow. Finally, any genes identified will be characterized, and evaluated as a candidate TSG. While the overall progress of our study has been considerable, and our ultimate objectives remain the same, we have made some necessary revisions to our original strategy during the last funding period. Our progress for the funding period from September 2001-August 2002 is outlined as follows.

Objective 1: Creation of GSE library (Task 1)

During the second year of funding we identified all clusters of partial cDNA clones (ESTs) and known genes, located within the target interval on 8p22, and completed construction of a GSE library of these clones. However, at the later stages of analysis, when large amounts of newly obtained EST and genomic sequence information became available, we came to the realization, that the majority of these EST clusters do not represent real genes, and most likely are not expressed. We have had an analogous experience with another interval that others are investigating in our laboratory on 5q31, which showed the same general trend, where the majority of EST clusters were cloning artifacts, parts of intronic sequences, or pseudogenes, rather than actual expressed genes. We have come to the conclusion, that at least for the particular MSR centered interval on 8p22, that continuation of work with a library containing unrelated sequences seems, for the most part, rather unjustified. This interval contains a very limited number of expressed sequences, which can be easier tested on individual basis. This led us to a conclusion that we have to re-consider our general approach for the selection of the cDNA clones for the library. In the previous summary report, we briefly discussed investigation of gene targets that have resulted from differential gene expression studies using GeneFilters arrays (Research Genetics). For the current funding period, we have expanded our studies in this direction of investigation to include a much larger subset of genes, using the Affymetrix GeneChip technology.

The whole genome oligonucleotide-based Affymetrix U95A arrays (HG-U95A) contain all full-length genes and represents a total of 12,625 sequences that have been previously characterized in terms of function or

disease associations. All hybridizations and data analysis were performed at the Core Genomics Facility at UIC. In brief, Biotin-labeled cRNA probes have been prepared from approximately 5-15 ug of total cellular RNA, using the standard GeneChip® eukaryotic target labeling protocol (Affymetrix, Santa Clara CA). Fluorescent probes were generated by reverse transcribing total RNA using an oligonucleotide-dT primer containing a T7 polymerase site. Amplification and labeling of the cDNA was achieved by carrying out an in vitro transcription reaction in the presence of biotinlated dNTPs, resulting in the linear amplification of the cDNA population (approximately 30-100-fold). The biotin-labeled cRNA probe generated from test and reference samples was then hybridized to separate oligonucleotide arrays, followed by binding to a streptavidin-conjugated fluorescent marker. Detection of bound probe was achieved following laser excitation of the fluorescent marker and scanning of the resultant emission spectra using a scanning confocal laser microscope (Probe Array Scan, Agilent Technologies). Absolute signal detection values as well as comparative expression levels, direction of the change (change call) and change p-values were calculated with the use of the MicroArray Suite 5.0 expression analysis software (Affymetrix, Santa Clara CA).

Objective 2: Introduction of library into target cells (Task 1)
We previously reported ongoing evaluation of one very promising gene from our differential studies in our functional assay system. In brief, the sense and anti-sense constructs for PMEPA1 were introduced into the parental and chromosome 8-containing MB231 cells. To assess the functional significance of this gene in vitro, the infected MB231 and HT29 cells were plated in soft agar. However, anti-sense experiments in the suppressed cell line and expression of sense constructs in the parental line did not show any difference compared to the empty vector controls.

Our current directions for library construction are described further in the following section. However, as the groundwork for library introduction into target cells has been completed (summary report 2001), this puts us in a very good position to proceed with the new set of genes.

Objective 3: Characterization of Genes (Task 2)
As discussed above, evaluation and characterization of our initial transcripts resulted in the realization that the majority of these EST clusters do not represent real genes, and most likely are not expressed. However, the majority of the genes contained on the U95 GeneChips are well characterized, and complete cDNA sequences are readily available for most.

Analysis of the GeneChip data revealed that a total of 1,949 were calculated to be differentially expressed between the hybrid and parental cell lines. Table 1 (see appendix) contains a list of genes whose expression levels changed four-fold and higher and which have the highest p-values for detection of change in expression levels. Priority will be given to those that are most highly differential, as well as those with the most interesting biologies.

Additionally, we also performed comparative expression analysis on the colon cancer cell line HT-29 and it's chromosome 8 containing derivative, which was also suppressed in soft agar clonicity and tumorigenicity. Of interest, we detected a number of transcripts equally up-or down-regulated in both breast and colon cancer derived hybrids. These genes are considered highly relevant to carcinogenesis, as they are related to suppression of more than one cancer type. Table 2 contains a list genes with the highest detection of change p-values.

Genes Differentially Expressed in Both Breast and Colon Carcinoma Cells

Unigene	Genbank	Gene	Description	Cytband	Change
Hs.283749	D37931	RNASE4	ribonuclease, RNase A family, 4	14q11.1	decrease
Hs.349470	AF044311	SNGG	gamma-synuclein gene (breast cancer-specific protein)	10q23.2	decrease
Hs.5338	AF037335	CA12	carbonic anhydrase XII	15q22	decrease
Hs.5344	AL050025	AP1G1	adaptor-related protein complex 1, gamma 1 subunit	16q23	decrease
Hs.90786	U83659	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	17q22	decrease
Hs.94806	AB028985	ABCA2	ATP-binding cassette, sub-family A (ABC1), member 2	9q34	decrease

Some of these genes represent particularly interesting targets for subsequent studies. Among them is carbonic anhydrase XII (CAXII), a transmembrane glycoprotein with an active extracellular domain that is overexpressed on cell surfaces of certain cancers, including breast cancer. Carbonic anhydrases influence intra- and extracellular pH and ion transport in varied biological processes. This gene may play a role in in acidifying the microinviroment of cancer cells and was shown to be associated with tumor invasiveness. This gene is demonstrates potential a target for chemotherapy. Another gene in this group of genes is synuclein-gamma (SNCG) or breast cancer-specific protein 1. Synuclein-gamma is a member of the synuclein family of proteins which are believed to be involved in the pathogenesis of neurogenerative disorders. High levels of SNCG have been identified in advanced breast carcinomas, suggesting a correlation between overexpression of SNCG and breast tumorigenesis.

In conclusion, as a result of our studies over the past funding period, we have identified a number of potential tumor suppressor genes, including genes on 8p, related to breast carcinogenesis. Due to unforeseen but necessary revisions of the originally proposed strategy, functional studies of these genes are currently not feasible within the scope of this proposal. However, they serve as a very promising starting point for future investigations, and as potential targets for diagnostics, therapy, and prognostics in breast cancer.

Training

During this past funding period, training has continued in the form of active participation in regular journal clubs, seminars, lab meetings, and workshops, as well as manuscript preparation. Journal club and lab meetings involve valuable training and practice in oral presentation, as well as critical review of scientific data. Attending seminars and workshops provide education in current findings, and new technologies. Finally, manuscript preparation provides very important training in scientific writing skills and presentation of data.

Key Accomplishments

- Identification of differentially expressed genes between suppressed MB231.X8 and malignant MB231 cells, which are very promising targets of investigation.
- Identification of a subset of highly relevant suppression-related genes differentially expressed in both breast and colon cancer models.
- Functional evaluation of PMEPA1 in parental/hybrid assay system.

Reportable Outcomes

Banerjee K, Arvieva ZH, Spanknebel KA, Usha L, Sharma TT, Liang J, Gomes I, Westbrook CA. Differential gene expression in malignant breast and colon cancer cells and their suppressed counterparts. Am. J. Human Genetics 69 (suppl): 271, 2000.

Banerjee K, Arbieva ZH, Usha L, Le TT, Liang J, Gomes I, Westbrook CA. Identification of downstream targets of the putative tumor suppressor gene on 8p by differential gene expression analysis. Proceedings, American Association for Cancer Research 42: 428, 2001.

Appendix

Table 1: Genes Differentially Expressed Between MB231.c and MB231.X8 Cells

Unigene	Gene	Cytband	Fold Change[1]	Change[2]
Hs.123114	cystatin SN	20p11.2	n/a	D
Hs.168383	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	19p13.3-p13.2	n/a	D
Hs.25817	BTB (POZ) domain containing 2	19p13.3	n/a	D
Hs.349123	major histocompatibility complex, class II, DR beta 1	6p21.3	n/a	D
Hs.943	natural killer cell transcript 4	16p13.3	n/a	D
Hs.90786	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	17q22	n/a	D
Hs.171992	protein tyrosine phosphatase, receptor type, J	11p11.2	n/a	D
Hs.77910	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	5p14-p13	n/a	D
Hs.5338	carbonic anhydrase XII	15q22	n/a	D
Hs.119597	stearoyl-CoA desaturase (delta-9-desaturase)	10q23-q24	n/a	D
Hs.288771	DKFZP586A0522 protein	12q13.12	n/a	D
Hs.118630	MAX-interacting protein 1	10q24-q25	n/a	D
Hs.22868	protein tyrosine phosphatase, non-receptor type 11	12q24.1	n/a	D
Hs.103042	microtubule-associated protein 1B	5q13	n/a	D
Hs.77690	RAB5B, member RAS oncogene family	12q13	n/a	D
Hs.173464	FK506-binding protein 8 (38kD)	19p12	n/a	D
Hs.7718	hypothetical protein FLJ22678	2q36.1	n/a	D
Hs.315463	interleukin 24	1q32	n/a	D
Hs.79362	retinoblastoma-like 2 (p130)	16q12.2	n/a	D
Hs.204238	lipocalin 2 (oncogene 24p3)	9q34	n/a	D
Hs.181046		17q21	n/a	D
Hs.300423	putative protein similar to nessy (Drosophila)	12p13	n/a	D
Hs.211586		19q13.2-q13.4	n/a	D
Hs.2399	matrix metalloproteinase 14 (membrane-inserted)	14q11-q12	n/a	D
Hs.75450	delta sleep inducing peptide, immunoreactor	Xp21.1-q25	n/a	D
Hs.118630	MAX-interacting protein 1	10q24-q25	n/a	D
Hs.23590		1p12	n/a	D
Hs.103042	microtubule-associated protein 1B	5q13	n/a	D
Hs.54900	serologically defined colon cancer antigen 1	14q22	n/a	MD
Hs.239926	sterol-C4-methyl oxidase-like	4q32-q34	**7.5**	D
Hs.239018	RAB11B, member RAS oncogene family	19p13.3	n/a	D
Hs.347326	intercellular adhesion molecule 2	17q23-q25	n/a	MD
Hs.158302	chromosome 1 open reading frame 1	1p36.3	n/a	D
Hs.332053	serum amyloid A1	11p15.1	**7.0**	D
Hs.5344	adaptor-related protein complex 1, gamma 1 subunit	16q23	n/a	D
Hs.75765	GRO2 oncogene	4q21	n/a	D
Hs.914	major histocompatibility complex, class II, DP alpha 1	6p21.3	n/a	D
Hs.337629	Homo sapiens mRNA; cDNA DKFZp434D115 (from clone DKFZp434D115)	5	n/a	D
Hs.42712	MAX protein	14q23	n/a	D

Hs.83393	cystatin E/M	11q13	**6.5**	D
Hs.301209	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 10	10p12	n/a	D
Hs.30985	pannexin 1	11cen-q12.1	n/a	D
Hs.24734	oxysterol binding protein	11q12-q13	n/a	D
Hs.243901	Homo sapiens mRNA; cDNA DKFZp564C1563 (from clone DKFZp564C1563)	8	n/a	D
Hs.75074	mitogen-activated protein kinase-activated protein kinase 2	1q32	n/a	D
Hs.305890	BCL2-like 1	20q11.21	n/a	D
Hs.238648	oncostatin M receptor	5p13.1	n/a	D
Hs.197764	thyroid transcription factor 1	14q13	n/a	D
Hs.93913	interleukin 6 (interferon, beta 2)	7p21	n/a	D
Hs.30209	KIAA0854 protein	8q24.13	n/a	D
Hs.287820	fibronectin 1	2q34	**5.7**	D
Hs.239926	sterol-C4-methyl oxidase-like	4q32-q34	**5.7**	D
Hs.226213	cytochrome P450, 51 (lanosterol 14-alpha-demethylase)	7q21.2-q21.3	**5.7**	D
Hs.289082	GM2 ganglioside activator protein	5q31.3-q33.1	n/a	D
Hs.81248	CUG triplet repeat, RNA-binding protein 1	11p11	n/a	D
Hs.69771	B-factor, properdin	6p21.3	n/a	D
Hs.82065	interleukin 6 signal transducer (gp130, oncostatin M receptor)	5q11	n/a	D
Hs.198248	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	9p13	n/a	D
Hs.32312	KIAA0356 gene product	17q21.32	n/a	D
Hs.76807	major histocompatibility complex, class II, DR alpha	6p21.3	n/a	D
Hs.226117	H1 histone family, member 0	22q13.1	n/a	D
Hs.6790	DnaJ (Hsp40) homolog, subfamily B, member 9	7q31	n/a	D
Hs.81412	lipin 1	2p21	**4.9**	D
Hs.90786	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	17q22	n/a	D
Hs.180338	tumor necrosis factor receptor superfamily, member 12 (translocating chain-association membrane protein)	1p36.2	n/a	D
Hs.198288	protein tyrosine phosphatase, receptor type, R	12q14.3	n/a	D
Hs.106070	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	n/a	D
Hs.78781	vascular endothelial growth factor B	11q13	n/a	D
Hs.624	interleukin 8	4q13-q21	n/a	D
Hs.89603	mucin 1, transmembrane	1q21	n/a	D
Hs.64639	glioma pathogenesis-related protein	12q15	n/a	D
Hs.181015	signal transducer and activator of transcription 6, interleukin-4 induced	12q13	n/a	D
Hs.343522	ATPase, Ca++ transporting, plasma membrane 4	1q25-q32	n/a	MD
Hs.7647	MYC-associated zinc finger protein (purine-binding transcription factor)	16p11.2	n/a	D
Hs.283749	ribonuclease, RNase A family, 4	14	n/a	D
Hs.283429	SMC (mouse) homolog, X chromosome	Xp11.22-p11.:	n/a	D
Hs.154672	methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	2p12	n/a	D
Hs.61635	six transmembrane epithelial antigen of the prostate	7q21	n/a	D
Hs.127799	baculoviral IAP repeat-containing 3	11q22	n/a	D
Hs.287820	fibronectin 1	2q34	**4.0**	D
Hs.184641	fatty acid desaturase 2	11q12-q13.1	n/a	D
Hs.1194	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	3p25-p24	n/a	D
Hs.336429	GABA(A) receptor-associated protein like 1	12p13.31	n/a	D

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